# Anti-depressant and anti-nociceptive effects of 1,4-benzodiazepine-2-ones based cholecystokinin ($CCK_2$) antagonists

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**ABSTRACT: Various 1,4-benzodiazepines were synthesized around a Diazepam, Oxazepam and the n-propyl-1,4-benzodiazepine template. SAR studies of $CCK_2$ binding affinity were performed and selected examples of each series were tested *in vivo* in mice. In addition to an anxiolytic effect, antidepressant effects were discovered using 8 standard CNS assays in mice. Finally, the cocomittant administration of the 1,4-benzodiazepine based CCK antagonists enhanced the response to pain with a low dose of morphine, significantly.**

***Key Words:*** 1,4-Benzodiazepine template, anxiolytics, antidepressant, anti-nociceptive agent, CCK-B antagonist, $CCK_2$

## Introduction

Cholecystokinin, which act as a neuromodulator/gut hormone and CCK-ligands, agonists as well as antagonists (*1*) have been extensively investigated as potential drug candidates (*2*). CCK-antagonists (*3*) were studied as growth inhibitors in certain forms of cancer (*4*), as anxiolytics (*5*), in the treatment of schizophrenia (*6*), satiety (*7*) and as anti-panic agents (*8*). An agonist, the shortened CCK tetrapeptide, was found to induce panic in patients and these effects were blocked by CCK antagonists (*9*).

A phase II trial of Devazepide, a potent and $CCK_1$ selective antagonist (*10*) has been recently completed (*11*) showing a significant enhancement of the effect of morphine in the treatment of chronic and severe pain (*12*).

Asperlicin, a microbial metabolite, was the first non-peptidal CCK antagonist and analogues thereof, containing an indolylmethyl substituent in the 3-position were studies as CCK ligands (*13*). The 1,4-benzodiazepine template was varied by a combinatorial solid phase synthesis (*14*) and was optimised in terms of CCK binding affinity (*15*). In the search for new CCK ligands, in which the 1,4-benzodiazepine structure was replaced by an achiral template, the tryptophan-indole moiety was selected as starting point (*16*).

Here, the systematic variation of the 1,4-benzodiazepine template (*17*) and the testing of reaction intermediates resulted in $CCK_2$ ligands (Figure 1), which were subsequently studied in mice.

Having realized the relevance of the CCK receptor in the treatment of pain (*18*) and depression (*19*), novel

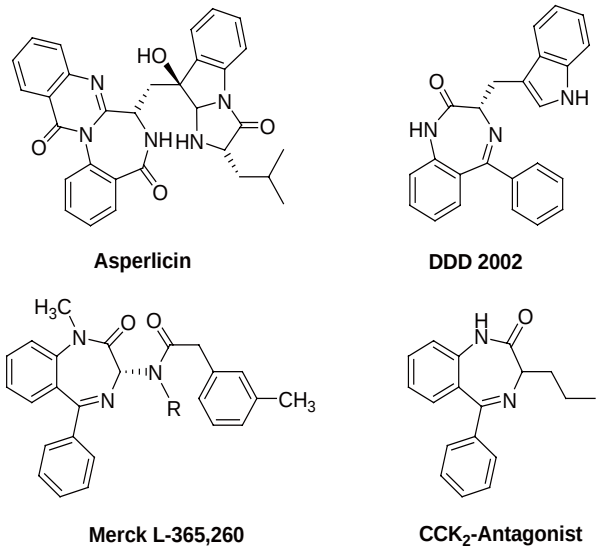


**Figure 1.** Chemical structures of Asperlicin, DDD 2002, Merck L-365,260, and $CCK_2$-antagonist.

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1,4-benzodiazepine based compounds were prepared and fully tested for these applications.

## Materials and Methods

### *Chemistry*

Atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) was carried out on a Hewlett-Packard 5989B quadrupole instrument connected to a 59987A unit with an APCI accessory. IR spectra were recorded as KBr discs on a Mattson 3000 FTIR spectrophotometer. Proton NMR spectra were obtained on a Bruker AC 250 instrument operating at 250MHz, with TMS as internal standard.

### *Experimental*

Synthesis of Diazepam **1** [Methyl-7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one]: 1.75 mL of Chloroacetyl chloride (22 mmol) was added under stirring to a solution of 2-amino-5-chloro-benzophenone (4.65 g, 20 mmol) in 25 mL of anhydrous ether at 0°C. The suspension containing the newly formed 2-chloroacetamido-5-chlorobenzophenone was stirred for half an hour at 0°C and for 2 h at room temperature. 64 mmoles of urotropine, 14.2 mL 2N aqueous HCl, 60 mL methanol and 5.8 mL water were added to this suspension and the mixture was heated under reflux for 16 h. It was cooled in an ice bath and the precipitate was filtered. The crystalls were washed with a 10 mL cold mixture of methanol/water. The product was dried at 60°C under reduced pressure. Yield 72% of Nor-desmethyldiazepam. APCI + m/s: m/z = 271; IR (KBr disc) $\upsilon_{max}$ = 3449, 3207, 2960, 1679, 1604, 1476, 1093, 794 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 4.31 (s, 2 H, C3:-$\underline{H}_2$), 7.1-7.65 (m, 8 H, arom. H), 10.0 p.p.m. (s,1 H, N1:-$\underline{H}$).

Conversion of Nor-desmethyldiazepam into Diazepam **1**: A mixture of 0.05 mole of Nor-desmethyldiazepam, 100 mL of *N,N*-dimethylformamide and 50% suspension of NaH in mineral oil (0.06 mol) was added in portions to a solution of the parent compound (0.05 mol) in DMF (100 mL). After stirring for 15 min at 0-5°C, methyliodide (0.06 mol) was added dropwise to the mixture with ice cooling and the solution was stirred for additional 30 min at RT. For work up, water was added and the suspension was extracted with EtOAc. The extract was washed with brine, dried ($Na_2SO_4$) and then concentrated under reduced pressure giving Diazepam in quantitative yield. The entitled compound is identical with the reference substance form Sigma-Aldrich. APCI + m/s: m/z = 285; TLC (Ether): $R_f$ = 0.4; $^1$H-NMR ($CDCl_3$) d 3.2 (s, 3H, N1:-C$\underline{H}_3$), 4.4 (s, 2H, C3:-$\underline{H}_2$), 7.1-7.6 p.p.m.(m, 8 arom. H).

### *Alkylation of Diazepam*

A solution containing 20 mL of anhydrous THF and 1.6 g (16 mmol) of diisopropylamine was cooled to -60°C and 11.2 mL (16 mmol) of 1.6 M *n*-butyllithium solution in hexane was added via syringe. The solution was stirred for 10-15 min and then warmed to room temperature. After the mixture was cooled back to -60°C, a 5 mL anhydrous DCM solution of Diazepam (2.3 g, 8 mmol) was added through a syringe and the reaction mixture was brought to room temperature over 30 min. The resulting dark red solution of the Diazepam anion was cooled to -20°C. 16 mmol alkylbromide was added in 5 mL of THF. The mixture was stirred and allowed to come to room temperature. The reaction was followed by TLC and for work up dilute HCl was added until pH 6-7. The reaction mixtures were extracted with ether (3 × 100 mL). The oil, obtained after evaporation of the dried etheral extract, was chromatographed. Elution with hexane-ethyl acetate (6:1) gave the desired compounds as oils.

*7-Chloro-1-methyl-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***2a***

APCI + m/s: m/z = 327; TLC (Ether): $R_f$ = 0.75; IR (KBr) $\upsilon_{max}$ = 2971, 2861, 1715, 1669, 1461, 1253, 1114, 1073, 738, 705 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.99 (t; 3H, *J* = 7Hz, C3:-$(CH_2)_2C\underline{H}_3$), 1.35-1.52 (m, 2H, C3:-$(CH_2)_2C\underline{H}_3$), 1.99 (m, 2H, C3:-$C\underline{H}_2CH_2CH_3$), 3.48 (s, 3H, N1:-C$\underline{H}_3$); 4.17 (t, 1H, *J* = 5Hz, C3:-$\underline{H}$), 7.2-7.87 (m, 8 arom. H), $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$(CH_2)_2\underline{C}H_3$), 19.1 (C3:-$CH_2\underline{C}H_2CH_3$), 28.9 (N1:-$\underline{C}H_3$), 35.0 (C3:-$\underline{C}H_2CH_2CH_3$), 68.0 (C-3), 122.6, 128.5, 129.5, 130.8, 131.3, 138.1, 141.2, 167.7, 171.7 p.p.m.

*3-Allyl-7-chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***2b***

APCI + m/s: m/z = 325 (80%), 311 (10%), 299 (5%), 285 (5%); TLC (Ether): $R_f$ = 0.75; IR (KBr) $\upsilon_{max}$ = 3436, 2923, 2856, 1668, 1607, 1484, 1386, 1320, 1263, 1094, 1031, 804, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 2.45 (m, 2 $\underline{H}$), 2.95 (s, 3 H, N1:-C$\underline{H}_3$), 3.85 (m, 1H, C3:-$\underline{H}$), 4.8-5.6 (br m, 3 H, C3:-$CH_2C\underline{H}$=C$\underline{H}_2$), 7.1-7.85 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 32.8 (N1:-$\underline{C}H_3$), 36.8, 62.1 (C-3), 122.2, 124.4, 127.1, 127.9, 129.4, 130.1, 131.7, 133.5, 136.4, 143.2, 167.4, 168.2 p.p.m.

*7-Chloro-1-methyl-5-phenyl-3-prop-2-ynyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***2c***

APCI + m/s: m/z = 323 (80%), 285 (15%), 241(5%); TLC (Ether): $R_f$ = 0.8; IR (KBr) $\upsilon_{max}$ = 3058, 2954, 2968, 1668, 1612, 1480, 1318, 1253, 1123, 819, 742, 701 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 2.0-2.4 (m, 3 H), 3.45 (s, 3 H, N1:-C$\underline{H}_3$), 5.7 (m, 1 H, C3:-$\underline{H}$), 7.2-7.75 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 21.9 (C3:-$\underline{C}H_2C{\equiv}CH$), 35.4 (N1:-

$\underline{C}H_3$), 122.3, 127.1, 128.0, 129.5, 130.8, 131.7, 136.5, 141.4, 166.5, 170.8 p.p.m.

*7-Nitro-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 3a via Sandmeyer reaction*

*p*-Nitrobenzophenone was reacted 1.15 eq of bromo-butyrylchloride at ambient temperature and the precipitate of *N*-(2-benzoyl-4-nitrophenyl)-2-bromopentanamide was filtered off. 0.02 mole of *N*-(2-benzoyl-4-nitrophenyl)-2-bromopentanamide containing potassium iodide as catalyst were dissolved in 100 mL of liquid ammonia. The mixture was stirred for 5 h in liquid ammonia and subsequently the ammonia was allowed to evaporate off at room temperature overnight. The residue was recrystallised in ether/petrolether. Yield 60%. APCI - m/s: m/z = 323 (75%), 241 (25%); TLC (Ether): $R_f$ = 0.6; IR (KBr disc) $\upsilon_{max}$ = 3449, 3338, 1640, 1611, 1476, 1313, 1297 (-$NO_2$), 1093, 958, 765, 699 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.98 (t; 3H, *J* = 7Hz, C3:-$(CH_2)_2C\underline{H}_3$), 1.25-1.9 (m, 4H, C3:-$(C\underline{H}_2)_2CH_3$), 3.48 (tr, 1H, *J* = 4Hz, C3:-$\underline{H}$), 7.2-7.75 (m, 6 arom. H), 7.9 (d, 1 arom. H), 8.1 (d, 1 arom. H), 8.5 (s, 1H, N1:-H); $^{13}$C-NMR ($CDCl_3$/MeOD) d 14.3 (C3:-$(CH_2)_2\underline{C}H_3$), 21.4, 35.4, 62.8 (C-3), 122.6, 128.5, 129.2, 129.5, 130.6, 131.4, 132.4, 137.3, 164.8, 168.9 172.8 p.p.m.

*7-Amino-5-phenyl-3-propyl-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-one **3b***

A solution of 3.2 mmole of 7-amino-3-propyl-benzodiazepine in 10mL of ethanol was added to an excess (15 mL) of a hot solution of 15% Sn-(II)-chloride in conc. hydrochloric acid. 25 mL of ethanol was added and the solution was filtered and concentrated in vacuum to give yellow needles, which were filtered and recrystallised from ethanol. APCI - m/s: m/z = 295 (80%), 241 (20%); TLC (Ether): $R_f$ = 0.45; IR (KBr) $\upsilon_{max}$ = 3432, 3262, 2960, 2859, 1646, 1532, 1498, 1318, 1241, 825, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.95 (s, 3H, C3:-$(CH_2)_2C\underline{H}_3$), 1.3-1.8 (two m, 4H, C3:-$(C\underline{H}_2)_2CH_3$), 2.4 (m, 2H), 3.65 (br s, 2H, C7:-$N\underline{H}_2$), 6.78 (s, 1H), 6.85 (d, 1H), 7.25-7.8 (m, 6 arom. H), 8.35 (d, 1H), 10.2 (s, 1H); $^{13}$C-NMR ($CDCl_3$) d 13.7 C3:-$(CH_2)_2\underline{C}H_3$), 22.3 (C3:-$CH_2\underline{C}H_2CH_3$), 27.6 (C3:-$\underline{C}H_2CH_2CH_3$), 37.8, 118.6, 120.6, 123.4, 125.1, 128.2, 129.2, 131.6, 138.4, 141.2, 171.9 p.p.m.

*7-Chloro-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one **3c***

A solution of 25 mmol of the amino-benzodiazepine **3b** in 30 mL of 6N hydrochloric acid was reacted with an aqueous solution of 30 mmol sodium nitrite (2.1 g) at 0-5°C. The resulting solution was added to 12 g copper chloride in 120 mL of 3N hydrochloric acid. The mixture was allowed to come to RT until the liberation of nitrogen was completed. After cooling the green solid, which has separated, was collected and dissolved in DCM. Copper salts were removed by washing the organic phase with aq. ammonia. It was dried with sodium sulfate, evaporated off and the desired compound was recrystallised from ethanol.

APCI + m/s: m/z = 313 (80%), 268 (15%), 264 (5%); mp: 197-198°C; TLC (Ether): $R_f$ = 0.72; IR (KBr disc) $\upsilon_{max}$ = 3218, 3123, 2923, 2851, 1687, 1604, 1476, 1320, 1220, 826, 699 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.99 (t; 3H, *J* = 7Hz, C3:-$(CH_2)_2C\underline{H}_3$), 1.3-1.5 (m, 2H, C3:-$CH_2C\underline{H}_2CH_3$), 2.23 (m, 2H, C3:-$C\underline{H}_2CH_2CH_3$), 3.53 (t, 1H, *J* = 5Hz, C3:-$\underline{H}$), 7.15-7.7 (m, 8 arom. H), 10.3 (s, 1H, N1:-H); $^{13}$C-NMR ($CDCl_3$) d 14.5 (C3:-$(CH_2)_2\underline{C}H_3$), 19.3 (C3:-$CH_2\underline{C}H_2CH_3$), 33.1 (C3:-$\underline{C}H_2CH_2CH_3$), 63.2 ($\underline{C}$3:-$(CH_2)_2CH_3$), 122.8, 124.9, 129.1, 130.2, 130.8, 131.6, 135.7, 137.4, 168.0, 172.5 p.p.m.

*General experimental for the N-Alkylation of 3-propyl-benzodiazepines*

The N1-alkyl/aryl derivatives (Table 1) were obtained by deprotonating the parent amide **3c** with 1.1 eq. sodium hydride in DMF followed by the reaction of the in situ formed anion with 1.2 eq. of the electrophile. The alkylation towards the methyl, ethyl, propyl and butyl derivatives **4a-4d** was carried out at 20°C. The alkylation with the electrophiles (Table 1) giving the phthalimide-, piperidine-derivatives **4g-4l** was carried out at 50°C.

The N1-hydroxylmethyl-benzodiazepine **4e** was obtained from formaldehyde, generated by thermal decomposition of paraformaldehyde. After 30 min the reactions were quenched with 10% aq. hydrochloric acid. The mixture was extracted with methylenchloride (3 times), concentrated in vacuum to give the N1 alkylated benzodiazepines. The crude product was purified further by solid extraction with ether.

*7-Chloro-1-ethyl-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one **4a***

APCI + m/s: m/z = 343 (90%), 268 (10%); TLC (Ether): $R_f$ = 0.85; IR (KBr) $\upsilon_{max}$ = 2990, 2863, 1723, 1673, 1602, 1482, 1255, 1124, 1080, 693 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 1.01 (t; 3H, *J* = 7Hz, C3: -$(CH_2)_2C\underline{H}_3$), 1.35-1.50 (m, 5H, C3:-$(CH_2C\underline{H}_2CH_3$ + N1:-$CH_2C\underline{H}_3$), 2.23 (m, 2H, C3:-$C\underline{H}_2CH_2CH_3$), 3.65 (q, 2H, N1:-$C\underline{H}_2CH_3$); 4.33 (t, 1H, *J* = 5Hz, C3:-$\underline{H}$), 7.2-7.87 (m, 8 arom. H), $^{13}$C-NMR ($CDCl_3$) d 13.3, 14.1, 22.9 (C3:-$CH_2\underline{C}H_2CH_3$), 28.2 (N1:-$\underline{C}H_2CH_3$), 35.0 (C3:-$\underline{C}H_2CH_2CH_3$), 68.0 (C-3), 122.6, 128.5, 129.5, 130.8, 131.3, 138.1, 141.2, 167.7, 171.7 p.p.m.

*7-Chloro-5-phenyl-1,3-dipropyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one **4b***

APCI + m/s: m/z = 356 (95%), 268 (5%); TLC (Ether): $R_f$ = 0.88; IR (KBr) $\upsilon_{max}$ = 2973, 2908, 1714, 1681, 1457, 1251, 1112, 1070, 738, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.73 (t, 3H, N1:-$CH_2CH_2C\underline{H}_3$), 1.0 (t; 3H, *J* = 7Hz, C3:-$(CH_2)_2C\underline{H}_3$), 1.43-1.7 (m, 6H, C3:-($C\underline{H}_2C\underline{H}_2CH_3$ + N1:-$CH_2C\underline{H}_2CH_3$), 3.48 (m, 2H, N1:-$C\underline{H}_2CH_2CH_3$), 4.25 (m, 1H, C3:-<u>H</u>), 7.26-7.87 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 10.8, 14.1, 19.3, 22.9, 48.4 (N1:-$\underline{C}H_2CH_2CH_3$), 63.5 (<u>C</u>3:-H), 123.7, 128.4, 128.7, 130.3, 130.8, 132.4, 140.9, 166.9, 167.7 p.p.m.

*1-Butyl-7-chloro-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4c***

APCI + m/s: m/z = 369 (90%), 279 (5%), (5%); TLC (Ether): $R_f$ = 0.9; IR (KBr) $\upsilon_{max}$ = 2924, 2867, 1717, 1683, 1461, 1283, 1121, 740, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.95-1.9 (m, 14H, C3:-($C\underline{H}_2C\underline{H}_2C\underline{H}_3$ + N1:-$CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_3$), 4.1-4.3 (m, 3H, C3:-<u>H</u> + N1:-$C\underline{H}_2CH_2CH_2CH_3$), 7.25-7.7 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 13.5, 13.9, 19.3, 19.6, 28.8, 38.6, 68.1 (<u>C</u>3:-H), 123.7, 128.3, 128.7, 129.3, 130.8, 132.4, 139.1, 140.5, 167.7, 169.7 p.p.m.

*1-Benzyl-7-chloro-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4d***

APCI + m/s: m/z = 403 (95%), 313 (5%); TLC (Ether): $R_f$ = 0.89; IR (KBr) $\upsilon_{max}$ = 2925, 2865, 1717, 1673, 1600, 1447, 1272, 1121, 738, 693 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.9-1.9 (m, 7H, C3:-($C\underline{H}_2C\underline{H}_2C\underline{H}_3$); 4.25 (m, 2H, N1:-$C\underline{H}_2$-Ph), 4.66 (m, 1H, C3:-<u>H</u>), 7.0-7.75 (m, 8 arom, H); $^{13}$C-NMR ($CDCl_3$) d 14.2 (C3:-$(CH_2)_2\underline{C}H_3$), 22.9 (C3:-$(CH_2\underline{C}H_2CH_3)$, 33.7 (C3:-$\underline{C}H_2CH_2CH_3$), 49.9 (N1:-$\underline{C}H_2$-Ph), 63.4 (<u>C</u>3:-H), 123.9, 127.3, 127.6, 127.8, 128.4, 130.4, 130.9, 131.2, 132.4, 136.5, 138.1, 140.3, 167.4, 169.7 p.p.m.

*7-Chloro-1-(hydroxymethyl)-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4e***

APCI + m/s: m/z = 343 (95%), 313 (5%); TLC (Ether): $R_f$ = 0.55; IR (KBr) $\upsilon_{max}$ = 3420, 2962, 2869, 1667, 1598, 1382, 1320, 1062, 825, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.99 (t, 3H, *J* = 7Hz, C3:-$CH_2CH_2C\underline{H}_3$); 1.2-1.7 (m, 4H, C3:-($C\underline{H}_2C\underline{H}_2CH_3$), 3.3-3.6 (br s, 1H, N1:-$CH_2$-O<u>H</u>), 4.2 (q, 1H, C3:-<u>H</u>), 7.0-7.5 (m, 8 arom, H); $^{13}$C-NMR ($CDCl_3$) d 14.3 (C3:-$CH_2CH_2\underline{C}H_3$), 17.9 (C3:-$CH_2\underline{C}H_2CH_3$), 29.1 (C3:-$\underline{C}H_2CH_2CH_3$), 69.2 (C-3), 96.0 N1:-$\underline{C}H_2$-OH), 121.5, 128.3, 128.5, 128.9, 130.4, 130.6, 132.2, 139.0, 141.8, 166.8, 169.3 p.p.m.

*7-Chloro-1-(3-hydroxypropyl)-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4f***

APCI + m/s: m/z = 371 (90%), 313 (10%); TLC (Ether): $R_f$ = 0.62; IR (KBr) $\upsilon_{max}$ = 3428, 2954, 2925, 1675, 1579, 1405, 1326, 1076, 823, 715, 676 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 1.0 (t, 3H, *J* = 7Hz, C3:-$CH_2CH_2C\underline{H}_3$), 1.2-2.4 (m, 6H), 3.1-3.8 (m, 4H), 4.5 (m, 1H, C3:-<u>H</u>), 7.25-7.55 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$CH_2CH_2\underline{C}H_3$), 19.3 (C3:-$CH_2\underline{C}H_2CH_3$), 30.8, 33.5 (C3:-$\underline{C}H_2CH_2CH_3$), 58.2, 63.5 (C-3), 97.2, 123.8, 128.4, 129.3, 130.2, 131.2, 136.2, 141.8, 167.4, 170.0 p.p.m.

*7-Chloro-5-phenyl-1-(2-piperidin-1-ylethyl)-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4g***

APCI + m/s: m/z = 424; TLC (Ether): $R_f$ = 0.85; IR (KBr) $\upsilon_{max}$ = 3443, 2928, 2866, 1675, 1608, 1476, 1315, 1112, 699 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.95 (t, 3H, *J* = 7Hz, C3:-$CH_2CH_2C\underline{H}_3$), 1.1-1.9 (m, 10H, N1:-$CH_2$-$CH_2$-<u>Pi--H</u> + C3:-$C\underline{H}_2C\underline{H}_2CH_3$), 2.1-2.4 (m, 2H), 2.65-2.8 (m, 4H, N1:-$CH_2$-$CH_2$-<u>Pi--H</u>), 3.46 (m, 1H), 4.2 (m, 1H), 4.51 (m, 1H, C3:-<u>H</u>), 7.25-7.60 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$CH_2CH_2\underline{C}H_3$), 19.2 (C3:-$CH_2\underline{C}H_2CH_3$), 22.3, 25.4, 30.8, 33.9 (C3:-$\underline{C}H_2CH_2CH_3$), 47.3, 53.9, 58.2, 63.5 (<u>C</u>3:-H), 127.3, 128.1, 130.4, 130.7, 131.4, 136.5, 141.5, 167.4, 169.6 p.p.m.

*2-[3-(7-Chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1H-1,4-benzodiazepin-1-yl)propyl]-1H-isoindole-1,3(2H)-dione* ***4h***

APCI + m/s: m/z = 500 (75%), 313 (20%), 268 (5%); TLC (Ether): $R_f$ = 0.88; IR (KBr) $\upsilon_{max}$ = 3467, 2960, 2869, 1771, 1710, 1677, 1602, 1462, 1400, 1372, 1037, 825, 726, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.98 (t, 3H, *J* = 7Hz, C3:-$CH_2CH_2C\underline{H}_3$), 1.1-2.4 (m, 6H, C3:-$C\underline{H}_2C\underline{H}_2CH_3$) + N1:-$CH_2$-$C\underline{H}_2$-$CH_2$-Phth), 3.4-3.95 (m, 4H, N1:-$C\underline{H}_2$-$CH_2$-$C\underline{H}_2$-Phth), 4.45 (m, 1H, C3:-<u>H</u>), 7.1-7.9 (m, 12 arom. H); $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$CH_2CH_2\underline{C}H_3$), 19.3 (C3:-$CH_2\underline{C}H_2CH_3$), 33.5, 35.2 (C3:-$\underline{C}H_2CH_2CH_3$), 44.8, 51.9, 63.4 (<u>C</u>3:-H), 123.3, 128.7, 129.6, 129.9, 130.2, 131.4, 131.9, 133.6, 136.2, 142.1, 166.5, 169.2 p.p.m.

*Ethyl 2-(7-chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1H-1,4-benzodiazepin-1-yl)acetate* ***4i***

APCI + m/s: m/z = 399 (90%), 313 (5%), 268 (5%); TLC (Ether): $R_f$ = 0.65; IR (KBr) $\upsilon_{max}$ = 2958, 2865, 1717, 1677, 1580, 1461, 1268, 1201, 1069, 823, 740, 703 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 0.9-1.1 (m, 6H, C3:-$CH_2CH_2C\underline{H}_3$ + N1:-$CH_2$-$CO_2CH_2C\underline{H}_3$), 1.2-1.85 (m, 4H, C3:-$C\underline{H}_2C\underline{H}_2CH_3$), 4.1-4.3 (m, 4H, N1:-$C\underline{H}_2$-$CO_2C\underline{H}_2CH_3$), 4.5 (q, 1H, *J* = 5Hz, C3:-<u>H</u>), 7.25-7.8 (m, 8 arom. H); $^{13}$C-NMR ($CDCl_3$) d 13.2 (N1:-$CH_2$-$CO_2CH_2\underline{C}H_3$), 14.0 (C3:-$CH_2CH_2\underline{C}H_3$), 19.6 (C3:-$CH_2\underline{C}H_2CH_3$), 33.6 (C3:-$\underline{C}H_2CH_2CH_3$), 49.2 (N1:-$\underline{C}H_2$-$CO_2CH_2CH_3$), 61.5, 63.8 (<u>C</u>3:-H), 122.8, 128.6, 128.9, 129.4, 130.6, 131.3, 136.4, 140.2, 166.9, 169.8 p.p.m.

*2-(7-Chloro-2-oxo-5-phenyl-3-propyl-2,3-dihydro-1H-1,4-benzodiazepin-1-l)acetonitrile* ***4j***

APCI + m/s: m/z = 352 (90%), 268 (10%); TLC (Ether): $R_f$ = 0.81; IR (KBr) $\upsilon_{max}$ = 2930, 2861, 2280 (N1:-$CH_2$-C≡N), 1723, 1687, 1598, 1324, 1265, 825, 744, 695 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 1.01 (t, 3H, *J* = 7Hz, C3:-$CH_2CH_2CH_3$), 1.1-1.9 (m, 4H, C3:-$CH_2CH_2CH_3$), 3.45 + 3.6 (two m, 2H, N1:-$CH_2$-C≡N), 4.5 (q, 1H, *J* = 5Hz, C3:-H), 7.1-7.8; 7.1-7.9 (m, 8 arom, H); $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$CH_2CH_2CH_3$), 19.3 (C3:-$CH_2CH_2CH_3$), 33.4 (C3:-$CH_2CH_2CH_3$), 38.9 (N1:-$CH_2$-C≡N), 62.9 (C3:-H), 115.4 (N1:-$CH_2$-C≡N), 122.8, 128.4, 128.9, 129.4, 130.4, 130.9, 131.5, 137.4, 139.2, 167.4, 169.3 p.p.m.

*7-Chloro-1-(2-oxo-2-phenylpropyl)-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***4k***

APCI + m/s: m/z = 431 (80%), 313 (10%), 279 (10%); TLC (Ether): $R_f$ = 0.82; IR (KBr) $\upsilon_{max}$ = 2969, 2871, 1717, 1688, 1598, 1465, 1255, 1116, 1076, 740, 693 $cm^{-1}$; $^1$H-NMR ($CDCl_3$) d 1.0 (t, 3H, *J* = 7Hz, C3:-($CH_2CH_2CH_3$); 1.25-1.9 (m, 4H, C3:-($CH_2CH_2CH_3$), 4.27 (m, 3H, C3:-H + N1:-$CH_2$-CO-Ph), 7.0 - 8.0 (m, 13 arom. H); $^{13}$C-NMR ($CDCl_3$) d 14.1 (C3:-$CH_2CH_2CH_3$), 22.3 (C3:-$CH_2CH_2CH_3$), 32.5 (C3:-$CH_2CH_2CH_3$), 53.3 (N1:-$CH_2$-CO-Ph), 63.8 (C-3), 123.2, 128.1, 128.7, 129.1, 130.9, 131.2, 131.5, 135.2, 136.9, 137.3, 139.9, 167.9, 169.0, 191.5 (N1:-$CH_2$-CO-Ph) p.p.m.

*7-chloro-1-(3,3-dimethyl-2-oxobutyl)-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***5a***

Alkylation method: A 50% suspension of NaH in mineral oil (0.06 mol) was added in drops to a solution of Oxazepam (0.05 mol) in dry DMF (100 mL). After stirring for 15 min at RT, the alkylating agent (0.06 mol) was added in drops to the mixture, with ice cooling. The solution was stirred for additional 30-45 min at RT. For work up water was added (75 mL) and the suspension was added to ethylacetate (75 mL). The extract was washed with brine (2 × 100 mL), dried over sodium sulphate and the solvent was evaporated. Column chromatography was performed with ether/petrolether 1:2 as eluent.

Yield: 81%. $R_f$ (ether/petrolether 1:2) = 0.51 Mol. Weight: 384.9 Mol. Formula: $C_{21}H_{21}ClN_2O_3$. MS (APCI(-)): 383, 385 (M-1), 285, 287 (M+) m/z. IR (KBr-disc) $\upsilon_{max}$: 3450, 2933, 2358, 1710, 1677, 1596, 1482, 1322, 1131 & 693 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) 300K: 1.23 (s, $(CH_3)_3$), 4.81 (s, C3-H), 5.04-5.12 (m, -$CH_2$-), 7.05-7.67 (m, Ar-9H) p.p.m. $^{13}$C-NMR (DMSO-$d_6$) 300K: 26.3 (($CH_3$)$_3$), 43.5 (($CCH_3$)$_3$), 53.2 ($CH_2$), 82.0 (C3), 122.9, 128.3 (2 × C), 128.4, 129.6, 129.8 (2 × C), 130.4, 130.8, 131.9, 137.4, 140.1 (Ar-C), 155.3 (C=O), 166.9 (C=N), 169.4 (C=O) p.p.m.

*7-chloro-3-hydroxy-5-phenyl-1-prop-2-ynyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one* ***5b***

Yield: 67%. $R_f$ (ether/petrolether 1:2) = 0.38 Mol. Weight: 324.8. Mol. Formula: $C_{18}H_{13}ClN_2O_2$. MS (APCI(-)): 323, 325 (M-1), 284, 286 (M+) m/z. IR (KBr-disc) $\upsilon_{max}$: 3418, 3291, 3225, 2923, 1700, 1634, 1478, 1415, 1324, 1131, 1002 & 695 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) 300K: 2.10-2.34 (t, CH, *J* = 24.7, 25.0Hz), 4.51-4.66 (m, -$CH_2$-), 5.04 (C3), 7.21-7.63 (Ar-H) p.p.m. $^{13}$C-NMR ($CDCl_3$) 300K: 37.0 (-$CH_2$-), 73.5, 75.19 (CH), 86.6 (C3), 123.4, 128.3 (2 × C), 128.3, 129.4 (2 × C), 130.3, 130.7, 131.1, 132.1, 137.1, 139.5 (Ar-H), 164.6 (C=O), 166.1 (C=N) p.p.m.

*Preparation of 7-Chloro-5-phenyl-3-propyl-1,3-dihydro-2H-1,4-benzodiazepin-2-thione* ***6***

1.8 g (6 mmol) of template **3c** and Lawesson's reagent (2.67 g, 6.6 mmol) were refluxed in 50 mL of pyridine. The reaction was completed after 8-10 h. The reaction mixture was cooled, concentrated in vacuum and the suspension of the residue in ice water was extracted with 50 mL of dichloromethane. The organic phase was filtered, dried and evaporated in vacuum. The crude product was further purified by column chromatography with ether.

Yield: 75%; MW 329; APCI + m/s: m/z = 329 (80%), 295 (10%), 268 (10%); mp: 238-242°C; TLC (ether): $R_f$ = 0.85; IR (KBr disc) $\upsilon_{max}$ = 3440, 2952, 1614, 1569, 1475, 1318, 1160, 1027, 828, 698 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) d (ppm) 0.9-1.7 (br m, 5H), 2.1-2.5 (m, 2H), 4.09 (m, 1 H, C3:-H), 7.2-7.6 (m, 8 arom. H), 11.6 (s, 1H, N1:-H). $^{13}$C-NMR ($CDCl_3$) d (ppm) 14.5 (C3:-$(CH_2)_2CH_3$), 19.5, (C3:-$CH_2CH_2CH_3$), 36.3 (C3:-$CH_2CH_2CH_3$), 67.1 (C-3), 124.9, 128.3, 128.9, 129.8, 130.2, 130.6, 131.8, 135.2, 137.8, 169.2, 202.3 (C=S).

*8-Chloro-1-methyl-6-phenyl-4-propyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine* ***7***

0.86 g (2.6 mmol) of template **2b** and acetylhydrazide (0.58 g, 7.8 mmol) was refluxed in 25 mL of *n*-butanol for 12 h to give the title product (0.68 g, 75%). The reaction mixture was cooled, concentrated in vacuum and the suspension of the residue in ice water was extracted with EtOAc (50 mL). The organic phase was dried with $K_2CO_3$, filtered, and evaporated in vacuum. The product was purified further by column chromatography with ether/10% methanol.

Yield: 34%; MW 351; APCI + m/s: m/z = 351; mp: 217-219°C; TLC (ether/10% methanol): $R_f$ = 0.45; IR (KBr disc) $\upsilon_{max}$ = 3420, 2925, 2865, 1604, 1531, 1482, 1424, 1301, 1096, 695 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) d (ppm) 1.0 (s, 3H, (C3:-$(CH_2)_2CH_3$), 1.4-1.9 (br m, 4H, C3:-$(CH_2)_2CH_3$), 2.61 (s, 3H, C1:-$CH_3$), 4.52 (s, 1H, C4:-H), 7.2-7.7 (m, 8 H, arom. H). $^{13}$C-NMR ($CDCl_3$) d (ppm) 12.1 (C4:-$(CH_2)_2CH_3$), 22.5, 31.7, 56.5 (C-4), 124.6, 128.3,

129.2, 130.7, 130.9, 131.2, 131.4, 132.1, 138.4, 165.9.

*Pharmacology*

*$^{131}$I-CCK-8 receptor binding assay*: The $CCK_1$ and $CCK_2$ receptor binding assays were performed by using guinea pig pancreas or guinea pig cerebral cortex, respectively. For the $CCK_2$ assay membranes from male guinea pig brain tissues were prepared according to the modification described by Saita *et al.* 1991. For the $CCK_1$ binding assay pancreatic membranes were obtained as described by Charpentier *et al.* 1988. All the binding assays were carried out in duplicate with L-365260 and Devazepide as internal standards.

In order to prepare the tissue the cerebral cortex was weighed after dissection and then homogenized in 25 mL ice cold 0.32 M sucrose for 15 strokes at 500 rpm. It was then centrifuged at 1,000 g (3,000 rpm) for 10 min. The supernatant was centrifuged at 20,000 g (13,000 rpm) for 20 min. This pellet was redispensed in the required volume of assay buffer as defined below with 5 strokes of homogenizer at 500 rpm. The final tissue concentration was 1 g original weight to 120 mL buffer. The tissue was stored in aliquots at -70°C.

For the receptor binding assay the radio ligand ($^{125}$I-Bolton Hunter labelled CCK, NEN) and the drugs to be tested were incubated at 25 pM with membranes (0.1 mg/mL) in assay buffer containing 20 mM Hepes, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl at pH 6.5 for 2 h at room temperature. The incubations were terminated by centrifugation. The membrane pellets were wasted twice with water and bound radioactivity was measured in a γ-counter.

The GABA-A binding assay (*20*) was performed with $^3$H-diazepam.

*Animal studies*

Experiments were conducted in male IRC mice obtained from the Animal House, Faculty of Medicine, Khon Kaen University. Each experimental group consisted of 6-8 animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University (HO 2434-76) accord with current UK legislation.

Mice were intraperitoneal injected with either test compound dissolved in 5% DMSO at the volume not more than 0.2 mL/animal. At 30 min after treatment, animals were tested as described in the following sections.

*Anxiolytic activity tests*

*The light/dark box*: Mice were placed in the light part of the light/dark box. The box was a Plexiglass cage, 25 × 50 × 20 cm, having one-third as a dark and two-third as a light compartment. A 40-W light bulb was used and positioned 10 cm above the center of the light component. The animals could walk freely between dark and light parts through the opening. The time animals spent in light part during the 5 min interval was recorded. The mouse was considered to be in the light part when its 4 legs were in the light part.

*The elevated plus-maze*: The wooden elevated plus-maze consisted of two open arms (30 × 10 cm) without any walls, two enclosed arms of the same size with 5-cm high side walls and end wall, and the central arena (10 × 10 cm) interconnecting all the arms. The maze was elevated approximately 30 cm height from the floor. At the beginning of the experiment the mouse was placed in the central arena facing one of the enclosed arm. During a 5 min interval, the time animals spent in the open arms of plus-maze was recorded. The mouse was considered to be in the open part when it had clearly crossed the line between the central arena and the open arm with its 4 legs.

*Nociception tests*

*The thermal tail-flick test:* The thermal response latency was measured by the tail flick test. The animals were placed into individual restraining cages leaving the tail hanging freely. The tail was immersed into water preset at 50°C. The response time, at which the animal reacted by withdrawing its tail from water, was recorded and the cut-off time was 10 sec in order to avoid damaging the animal's tissue.

*The hot plate test:* Mice were placed on a hot plate that was thermostatically maintained at 50°C. A Plexiglass box was used to confine the animal to the hot plate. The reaction time of each animal (either paw licking or jumping) was considered a pain response. The latency to reaction was recorded. For prevention of heat injury, the cut-off time of the test was 30 sec.

*Antidepression tests*

*The tail suspension test:* Mice were hung by their tail on the tail hanger using sticky tape for tail fixation, at approximately 1 cm from the end. The hanger was fixed in the black plastic box (20 × 20 × 45 cm) with the opening at the top front. The distance between the hanger to floor was approximately 40 cm. The mouse was suspended in the air by its tail and the immobile time was recorded during the period of 5 min. The duration of immobility was defined as the absence of all movement except for those required for respiration

*The forced swim test:* The forced swim test was carried out in a glass cylinder (20 cm diameter, 30 cm height) filled with water to the height of 20 cm. The water temperature was approximately 25-28°C. Mice were gently placed into the water and the immobility time was recorded by an observer during the period of 5 min. Immobility was defined as absence of all

movement and remained floating passively in the water with its head just above the water surface.

*Motor activity tests*

*The rota-rod test:* Mouse was placed on the rotating drum with the acceleration speed (Acceler. Rota-rod, Jones & Roberts, for mice 7650, Ugo Basile, Italy). The time animal spent on the rod is recorded.

*The wire mesh grasping test:* Mouse was placed on a wire mesh (20 × 30 cm). After a few seconds, the mesh was turned 180° and the time animal hold on the mesh was recorded.

*Potentiation of morphine induced-analgesia*

Each mouse received 2 injections. For the first injection, either 5% DMSO or synthetic CCK antagonists was injected intraperitoneally. Twenty min after the first injection, either normal saline or various doses of morphine were injected subcutaneously as the second injection.

The thermal response latency was measured by the tail flick test. The base line withdrawal thresholds (BT) were recorded prior to the first injection. Test thresholds (TT) were measured 60 min after the second injection. The cut off time was set to 45 sec. This was to avoid any tissue damage to the paw during the course of analgesia testing. The test thresholds were expressed as a percentage of Maximal Possible Effect (%MPE) using the equation: $\%MPE = \{(TT-BT)/(45-BT)\} \times 100$

*Statistical methods*

The data were expressed as mean ± SD and one-way analysis of variance (ANOVA) and supplementary Tukey test for pairwise comparison were tested to determine for any significant difference at $p < 0.05$.

## Results and Discussion

*Chemistry*

Nor-methyldiazepam was synthesised according to the standard literature procedure (*21*) starting with aminoketones and chloroacetylchloride (*22*). Subsequent imine formation of the 7 membered ring system with urotropine, via the known α-amino intermediate, gave the target compound in 87% yield. Alkylation of the 1,4-benzodiazepin-2-one with dimethylsulfate gave Diazepam **1**, which was used as template for alkylation reactions and condensations with propionaldehyde in the 3-position (*23*).

The Diazepam **1** was deprotonated with LDA at -78°C and the alkylation with propylbromide, allylbromide and propargylbromide as electrophile with a catalytic amount of KI gave the alkylated benzodiazepines **2b-2c** in THF at ambient temperature over night (*24*) (Scheme 1).

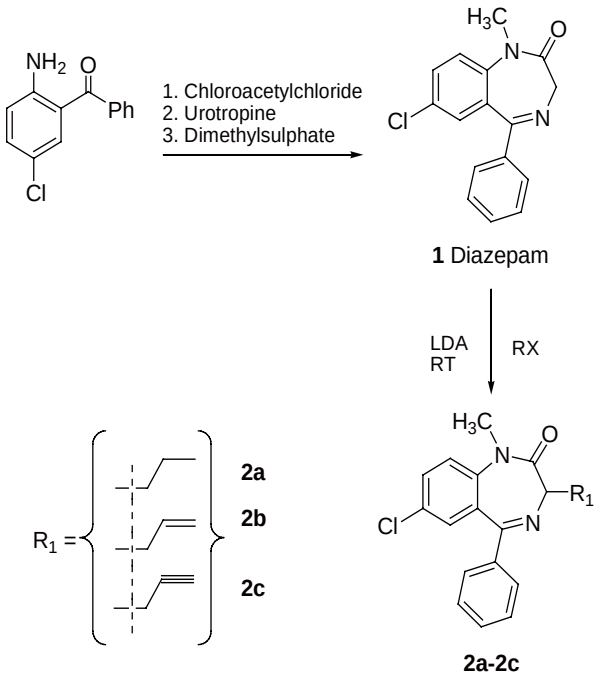
In Scheme 2 a novel synthesis suitable for large scale preparation of the 3-propyl-1,4-benzodiazepine **3c** and the further alkylation products **4a-4k** are outlined. The route was adapted from the synthesis of Diazepam, in which bromo-butyric acid chloride was reacted to form the desired amide **I.** Bromobutyric acid chloride was obtained from the readily available bromobutyric acid with thionyl chloride. Starting material nitro-benzophenone was reacted with bromo-butyric acid chloride to give the desired amide **I** which was isolated. The treatment of this benzamide **I** with ammonia gave the 3-propyl-1,4-benzodiazepine **3a** in good yield. Unlike for the synthesis of Diazepam, Urotropine could not be used to the formation of the 7-membered ring system.

The nitro-benzodiazepine **3a** was reduced with a solution of Sn-(II)-chloride to the amino-benzodiazepine **3b**, which was converted into chloride **3c** in a Sandmeyer reaction. The amine **3b** was diazotated in situ with cupper chloride under acetic conditions.

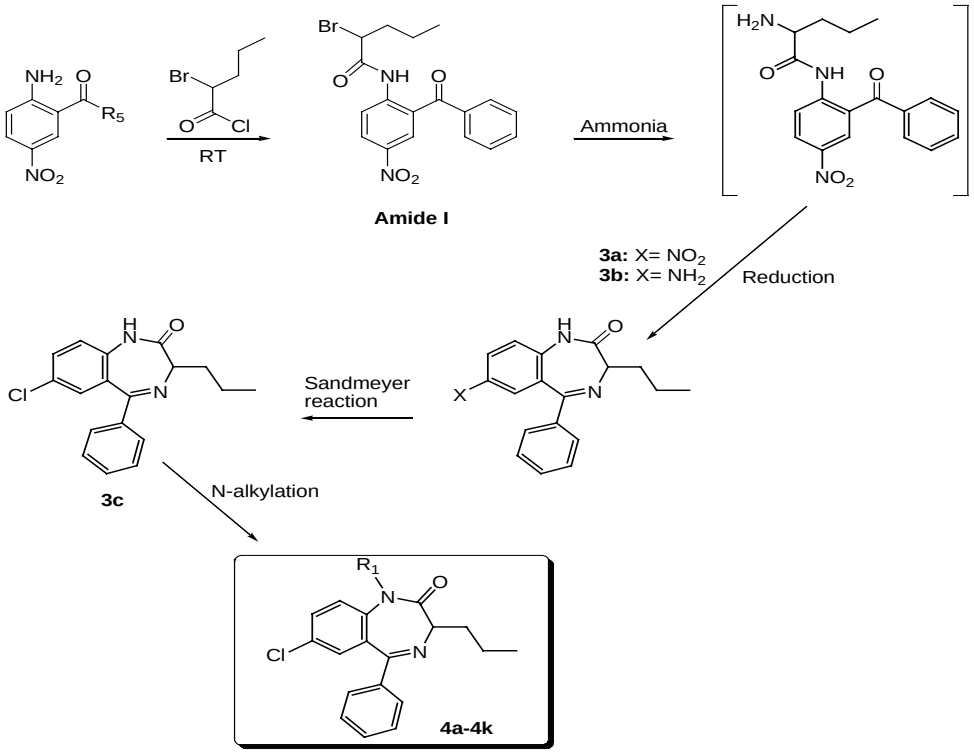
The *N*-alkylated benzodiazepines **4a-4k** were synthesised from the benzodiazepine template **3c** with sodium hydride in DMF (*25*). No dialkylation products were obtained and no comumn chromatography was required for further purification.

Further *N*-alkylated benzodiazepines were synthesized using the Oxazepam template under same reaction conditions. With allylbromide and 3,3-dimethyloxobutyl chloride the Oxazepam derivatives **5a** and **5b** were obtained.

The preparation of the 4H-[1,2,4]triazolo[4,3-a]-1,4-benzodiazepine **7** via the thioamide intermediate **6** is outlined in the synthetic Scheme 4.




**Scheme 1.** *N*-Alkylation of Diazepam.




**Scheme 2.** *N*-alkylation of 3-propyl-1,4-benzodiazepines.

The template **3c** was converted into thioamide **6** with tetraphosphorus decasulfide (P4S10) under reflux conditions. In an improved method, tetraphosphorus decasulfide was replaced by with 2,4-bis(4-methoxyphenyl)-2-4-dithioxo-1,2,3,4-dithiadiphosphetane (Lawesson's reagent) (*26*) which gave the thioamide **6** in 75% yield.

The activated thioamide **6** was reacted with an excess of acetylhydrazide into the desired 1,2,4-triazolo-1,4-benzodiazepines **7**. *N*-butanol was essential to achieve a high reflux temperature for this conversion.

The Nva template **8** was prepared by refluxing 5-chloro-2-aminobenzophenone with 2 equivalent of *L*-Nor-valine ethylester HCl for 48 h in presence of a catalytic amount of DMAP.

*X-ray analysis*

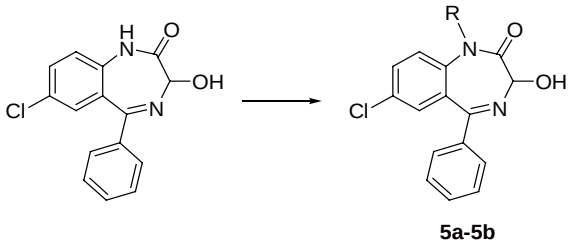
As gold standard of characterization, the X-ray structure of the *N*-alkylated propyl-benzodiazepine **4i** is outlined in Figure 2.

It was found as expected, that the benzodiazepine **4i** ring was not part of a flat extended system. Torsion angles within this ring about bonds that have full or partial double bond character (N4-C5, C10-C11, N1-C2) did not exceed 4.2(4)° in magnitude. Torsion angles within the ring about the intervening bonds N1-C10 and C5-C11 attained the intermediate values of -42.3(4) and 44.2(4)°, respectively. Those about C2-C3 and C3-N4 at the opposite end of the ring had the largest magnitudes, being 78.2(3) and -73.2(3)°. The amide resonance affecting atoms N1, C2 and O17 were confirmed by the elongation of the C2-O17 distance to 1.217(3) Å compared with 1.197(4) Å for the ester C13-O18. The partial negative charge on O17 helped to attract a C23-H23…O17 hydrogen bond with H…O distance 2.48 Å and C-H…O angle 165°. Structural details were listed in Table 1.

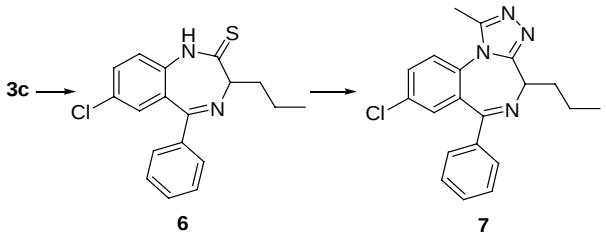
*SAR-studies - binding affinity*

For the entire set of 1,4-benzodiazepines the chemical yields and the binding affinities were outlined for the $CCK_2$ receptor in Table 2.

Alkylated derivatives of diazepam (**2a-2c**) were




**Scheme 3.** *N*-Alkylation of Oxazepam.




**Scheme 4.** Preparation of triazolo-propylbenzodiazepine **7**.

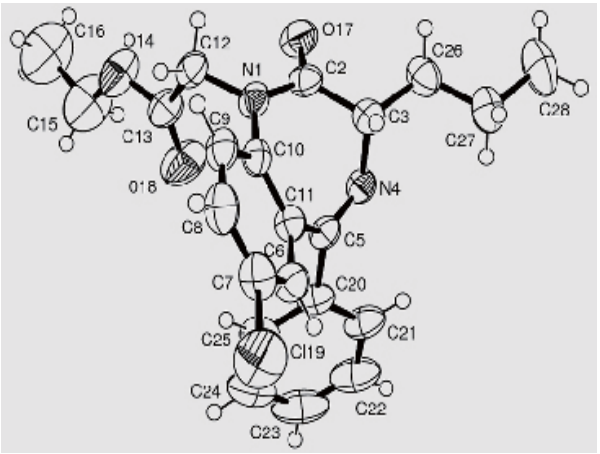



**Figure 2.** X-ray structure of benzodiazepine **4i**.

found to occur a very low binding affinity towards the $CCK_2$ receptor.

Reaction intermediate **3c** showed a good potency with an $IC_{50}$ about 300 nM, while the precursors, the nitro-intermediate **3a**, and the amino-intermeate **3b** were relatively biologically inactive.

The ligand **3c** containing a C3 unit in the 3-position of the 1,4-benzodiazepine was previously found to bind best to a postulated lipophilic pocket at the $CCK_2$ receptor (*13*). In order to optimize the affinity of the 3-propyl-1,4-benzodiazepines, additional substituents were introduced in the N1-position giving the series of *N*-alkylated propylbenzodiazepines **4a-4k,** of which the nitrile **4j** occurred an enhanced binding affinity compared with parent derivative **3c**.

The more complex derivatives such as the piperidino-, and phthalimido-benzodiazepines **4g**, **4f** and **4h** did not show an enhanced binding affinity at the CCK receptor.

As previously reported for the 3-ureido-benzodiazepines, N1-alkylation (*27*) should have shown enhanced binding on the $CCK_2$ receptor, but with the majority of the electrophiles used here, the affinity was significantly lower than for the N1-unalkylated 1,4-benzodiazepine **3c**.

Functionalised hydrophilic substituents at the N1 position as seen for the hydroxy-alkyl derivatives **4e**, **4f** resulted in the loss of binding affinity.

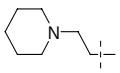
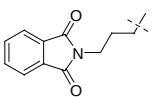
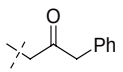
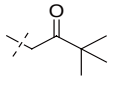
In order to gain a high chemical diversity an amide **6u**, an ester **4i** and a ketone **4k** was formed of the 1,4-benzodiazepine template.

A decreased affinity was observed for the N1 alkylated propyl-1,4-benzodiazepine **4k** unlike Merck's 3-ureido-1,4-benzo-diazepines, which have previously shown an increased binding with this N1-substituent (*28*).

**Table 1.** Crystal structure determination for benzodiazepine **4i**

| | |
|---|---|
| Empirical formula | $C_{22}H_{23}ClN_2O_3$ |
| a/Å | 9.2332(8) |
| b/Å | 17.702(4) |
| c/Å | 13.052(5) |
| β/° | 97.466(15) |
| Z | 4 |
| Calculated density/g·cm$^{-3}$ | 1.253 |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| Diffractometer | Enraf-Nonius CAD4 |
| Radiation used | Mo-Kα |
| Monochromator | Graphite |
| Crystal size/mm | 0.45 × 0.20 × 0.05 |
| Temperature/K | 294(2) |
| Data collection mode | ω-2θ scans |
| Theta range/° | 2.22-24.98 |
| Reciprocal lattice segments | $-10 \le h \le 10$<br>$-11 \le k \le 21$<br>$0 \le l \le 15$ |
| Reflections measured | 6512 |
| Symmetry-independent reflections | 3685 |
| Cut-off criterion | $I > 2\sigma(I)$ |
| Linear absorption coefficient/mm$^{-1}$ | 0.205 |
| Method of absorption correction | psi-scan |
| Method of solution | direct |
| Method of refinement | full-matrix LS |
| Final *R*(obs) | 0.0449 |
| Final *wR2* (all data) | 0.1478 |
| Residual density/e Å$^{-3}$ | -0.216, 0.275 |

**Table 2.** Chemical yields and $IC_{50}$ receptor binding data on the Cholecystokinin ($CCK_2$) receptor of the 1,4-benzodiazepines. (Standard: Merck L-365260 10 nM)

| Entry | Substituent | Yield (%) | $CCK_2$ (μM) |
|---|---|---|---|
| **1** | Diazepam | | |
| **2a** | $R_1$= propyl | 28 | 5 |
| **2b** | $R_1$= allyl | 35 | 2.5 |
| **2c** | $R_1$= propargyl | 22 | 2.2 |
| **3a** | X=$NO_2$ | 60 | 8 |
| **3b** | X=$NH_2$ | 65 | >50 |
| **3c** | X=Cl | 52 | 0.3 ± 0.01 |
| **4a** | $R_1$= ethyl | 78 | 1.2 ± 0.6 |
| **4b** | $R_1$= propyl | 74 | 5 ± 2 |
| **4c** | $R_1$= butyl | 65 | 3 ± 1 |
| **4d** | $R_1$= benzyl | 65 | 35 ± 3 |
| **4e** | $R_1$= hydroxymethyl | 21 | 37 ± 11 |
| **4f** | $R_1$= hydroxypropyl | 31 | 71 ± 21 |
| **4g** | $R_1$=  | 82 | 88 ± 12 |
| **4h** | $R_1$=  | 87 | 34 ± 6 |
| **4i** | $R_1$=  | 63 | 3.5 ± 0.4 |
| **4j** | $R_1$= cyanomethy | 53 | 0.19 ± 0.02 |
| **4k** | $R_1$=  | 21 | 4 ± 1.5 |
| **5a** | R=  | 81 | 0.19 ± 0.02 |
| **5b** | R= allyl | 67 | 0.98 |
| **6** | thioamide- | 56 | 0.20 ± 0.02 |
| **7** | triazolo- | 34 | 32 |
| **8** | L-isomer | 69 | 0.17 ± 0.02 |

A cyanomethyl substituent on the N1 position is desirable, firstly to enhance the affinity and secondly to differentiate the binding profile between the cholecystokinin and the benzodiazepine receptor. It is known that most of the substiuents in the N1 position block the affinity on the GABA-A receptor (*20*).

The alkylated derivatives based on the Oxazepam structure, such as **5a** and **5b** showed potent and moderate binding affinity. Compared to Oxazepam the binding affinity was increseased 100 fold for ketone **5a**.

The biosteric modification from amide **3c** into thioamide **6** gained some binding affinity, which was lost with the indroduction of the triazolo ring system, cp **7**.

The optically pure *L*-stereoisomere **8** of the *n*-propyl derivative displayed a further improved activity and is considered a promising example of an optically and chemically pure compound derived from nor-valine in only one chemical step (Figure 3).

In Figure 3, important structures were highlighted additionally for clarity. The crystalline **4i** was suitable for X-ray analysis, but lacked of binding affinity.

Nitrile **4j** displayed the best binding affinity of the *N*-alkylated propyl series **4**. The *t*-butyl–keton side chain decreased affinity of the *n*-propyl derivatives in the **4**-series, but enhanced the affinity for the Oxazepam template as seen as for Merck' ureas.

Thioamide **6** was found of improved CCK binding affinity. The triazolo-compound **7** was supposed a GABAA ligand, possibly with a modified subtype specificity, due to the 3-propyl substituent, but was not further investigated. The *L*-propyl-1,4-benzodiazepine derivative **8** combined control of stereochemistry at the C3 centre, easy access and good $CCK_2$ binding affinity.

*In vivo studies*

The neuropharmacological effects of intermediate **3c**, the active and inactive dpropylbenzodiazepines **4i** and **4j**, the *N*-alkylated Oxazepam analogue **5a**, thioamide **6** and the *L*-propyl benzodiazepine **8** were evaluated in mice in 8 different *in vivo* assays, compared to Diazepam **1** and desimipramine as standards.

The benzodiazepine **4j**, **5a**, **6** and **8** displayed equivalent potency for the $CCK_2$ receptor. All test compounds have been found inactive in the evaluation of pain in the hot plate and the tail flick assays (*29*), when administered as a single agent (Table 3).

The first step of the *in vivo* evaluation (*30*), was the determination of the MED, minimum effective dose, to select *in vivo* active compounds and to compare the results with the receptor binding data. Compounds without binding affinity were not found different from the control (propylen glycol) at doses of 0.1, 0.5, 1.0, 2.0, 5.0 and 10 mg. For example, **4i** displayed no binding affinity and was found inactive in mice.

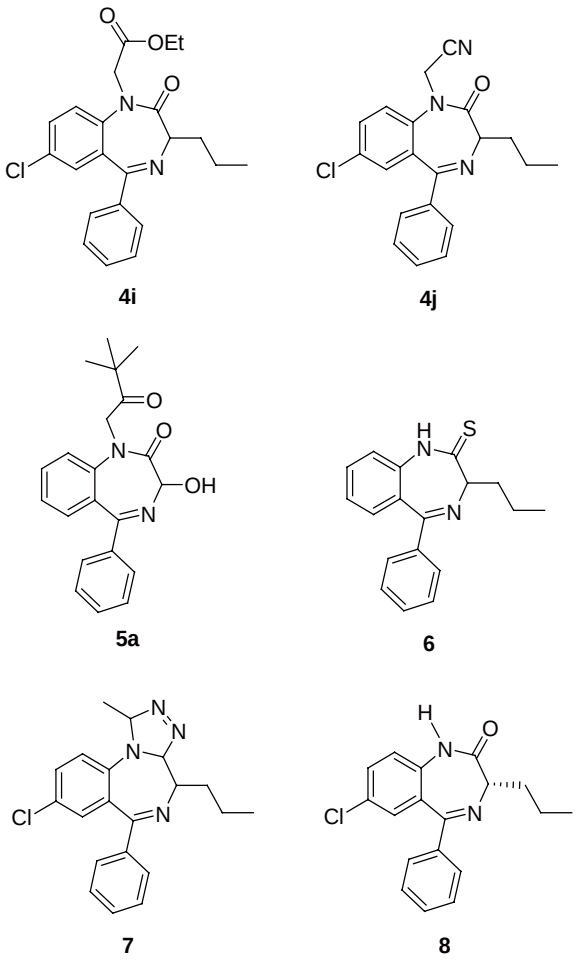



**Figure 3.** Important structures highlighted additional for the SAR-studies - binding affinity.

Benzodiazepine **4j**, **5a**, **6** and **8**, ligands of the same binding profile, had an MED of 1 mg/kg in both antidepressant assays and 2.5 mg/kg in the selected anxiolytic assays. The anxiolytic effect was evaluated by using the black and white box test (*31*) and the elevated x-maze (*32*) as two standard anxiolytic assays (Table 4).

Subsequently, full data of the equipotent $CCK_2$ selective derivatives **4j**, **5a** and **8** were collected and the $ED_{50}$ were calculated

*Anxiolytic assays:* Animals have shown at high doses in the black and white test (*33*) a significantly increased preference for the light area, and also the number of crossings between the two chambers were enhanced for **4j**, **5a** and **8**. An enhanced locomotor activity was determinated with an $ED_{50}$ of 9/11/7 mg/kg for the set of benzodiazepine derivatives. This correlated with the results of *the elevated plus maze test (X-maze)* (*34*), in which a greatly enhanced exploration of the open arms with an increased number of total crossings was observed. The anxiolytic effect is linked with $CCK_2$ binding affinity. The ureas (*19*) displayed as mixed CCK ligands, anxiolytic- and antidepressant effects at a similar low dose. The binding affinity correlated in this comparison well with *in vivo* results.

**Table 3.** *In vivo* evaluation of selected 1,4-benzodiazepines

| Compound | Receptor binding $IC_{50}$ (nM) | | Elevated plus-maze | Light/dark box | Tail suspension test | Forced swim test | Thermal tail flick test | Hot plate test | Rota-rod test | Wire mesh grasping |
|---|---|---|---|---|---|---|---|---|---|---|
| | $CCK_1$ | $CCK_2$ | | | | | | | | |
| **1** | >100000 | >100000 | 1 | 1 | - | - | - | - | 1 | 1 |
| **3c** | >10000 | 300 ± 10 | 5 | 5 | 2.5 | 2.5 | - | - | - | - |
| **4i** | >10000 | 3500 ± 400 | NS | NS | NS | NS | NS | NS | NS | NS |
| **4j** | >10000 | 190 ± 20 | 2.5 | 2.5 | 1 | 1 | - | - | - | - |
| **5a** | >10000 | 190 ± 10 | 2.5 | 2.5 | 1 | 1 | - | - | - | - |
| **6** | >10000 | 200 ± 10 | 2.5 | 2.5 | 1 | 1 | - | - | - | - |
| **8** | >10000 | 170 ± 10 | 2.5 | 2.5 | 1 | 1 | - | - | - | - |

NS: no significance could be observed at 0.1, 0.5, 1.0, 2.5, 5.0 and 10 mg/kg compared to the control.
MED: minimum effective dose (mg/kg) given in above table.

**Table 4.** *In vivo* studies of selected CCK antagonists in mice

| | $ED_{50}$ (mg/kg ± 1 mg/kg) | | |
|---|---|---|---|
| | **4j** | **5a** | **8** |
| Elevated plus maze | 9 | 11 | 9 |
| Light/dark box | 9 | 11 | 7 |
| Tail suspension test | 2 | 4 | 2 |
| Forced swim test | 2 | 3 | 2 |
| Thermal tail flick test | >100 | >100 | >100 |
| Hot plate | >100 | >100 | >100 |
| Rota rod test | >100 | >100 | >100 |
| Wire mesh grasping | >100 | >100 | >100 |

*Antidepressant assays:* Antidepressant drugs have the effect of reducing the duration of immobility in *the despair swim test (immobility time test)* (*35*). The set of ligands **4j**, **5a** and **8** decreased the immobility time at a very low dose and the $ED_{50}$ was calculated at 2/3/2 mg/kg. In the tail suspension test, which is based on a similar underlying mechanism, an $ED_{50}$ of 2/4/2 mg/kg was determined for **4j**, **5a** and **8**. Desipramine, a tricyclic antidepressant served as positive control, which occured a similar potency and magnitude of the antidepressant effect.

*Nociception and motor activity tests*: In all treated groups, no effect on nociception (*36*) was observed in the tail immersion test (*37*) and the hot plate method. An impairment of motor activity could not be observed in all tested models up to a dose of 100 mg/kg in the wire mash grasping and the rota rod test.

*Potentiation effect of **4j** and **8** on morphine-induced analgesia in mice*

CCK antagonists are supposed to enhance the effect of morphine and therefore the cocomittant administration of **4j** and **8** at a dose of 5 mg/kg body weight was investigated. Assays were carried out as described in experimental section. The thermal response latency of the animals were determined by the tail flick test and the results were expressed as %MPE = {(TT-BT)/(45-BT)} × 100.

Although no intrinsic analgesic effect of **4j** and **8** was observed as single agent, both CCK antagonists

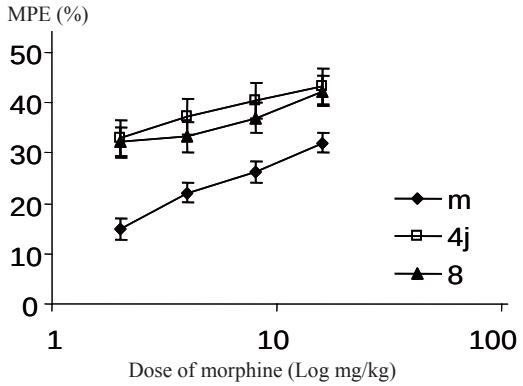



**Figure 4.** Potentiation effects of **4j** and **8** on morphine-induced analgesia in mice. Mice were injected with 5% DMSO or **4j** or **8** (5 mg/kg BW) (ip) and normal saline (sc) or morphine (sc) (2, 4, 8 or 16 mg/kg BW). There were 8-10 mice in each group and % maximum possible effect (MPE) was expressed as mean ± SD. **P*-value < 0.05 when compared to the control group (Note: **5a** not significantly different from **4j** and **8**). m, morphine; 4j, morphine + 5 mg/kg benzodiazepine **4j**; 8, morphine + 5 mg/kg benzodiazepine **8**.

increased the % MPE in response to morphine, at all doses of morphine tested significantly (Figure 4).

The best enhancement was found at a low dose such as 2 mg/kg of morphine and full investigation of this class of $CCK_2$ antagonist, further mixed and $CCK_1$ selective antagonists are in high progress.

As expected no significant difference between the benzodiazepine **5a/4j** and **8** was observed. Overall the dose response curve of morphine was shifted to the left indicating in general a potentiation of the analgesic effects of morphine.

## Conclusions

The 3-propylbenzodiazepine **3c** was firstly synthesized by a combinatorial approach, but due to the small scale no further evaluation could be performed. A new chemical approach toward **3c** provided the parent compound in good quantities and new *N*-alkylated analogues were derived thereof. This was completed by a third stereoselective on-step-synthesis of active **8.**

Based on the Oxazepam template an equipotent

N1-alkylated derivative was tested and developed further.

The structurally well known class of non-toxic benzodiazepies displayed as $CCK_2$ antagonist, antidepressant properties and the dose response curve of morphine was found to be significantly shifted to the left.

Studies are ongoing to evaluate the importance of mixed, $CCK_2$ or $CCK_1$ antagonists on the anti-nociceptive effects in conjunction with opiate agonists.

**References**


1. McDonald IM. $CCK_2$ receptor antagonists. Expert Opin Ther Pat 2001;11:445-462.
2. Satoh M, Kondoh Y, Okamoto Y, Nishida A, Miyata K, Ohta M, Mase T, Murase K. New 1,4-benzodiazepin-2-one derivatives as gastrin/cholecystokinin-B antagonists. Chem Pharm Bull 1995;43:2159-2176.
3. Bock MG, DiPardo RM, Mellin EC, Newton NC. Second generation benzodiazepine CCK-B antagonists. Development of subnanomolar analogues with selectivity and water solubility. J Med Chem 1994;37:722-724.
4. Lattmann E, Arayarat P. From CNS-drugs to anti-neoplastic agents: Cholecysto-kinin (CCK)-antagonists as modern anti-cancer agents. Science (KKU) 2003;31:178-193.
5. Hughes J, Woodruff GN. Neuropeptides function and clinical applications. Arzneimittelforschung 1992;42:250-255.
6. Rasmussen K, Stockton ME, Czachura JF, Howbert JJ. Cholecystokinin (CCK) and schizophrenia: the selective CCKB antagonist LY262691 decreases midbrain dopamine unit activity. Eur J Pharmacol 1991; 209:135-138.
7. Dourish CT, Rycroft W, Iversen SD. Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. Science 1989;245:1509-1511.
8. Trivedi K, Bharat J. Cholecystokinin receptor antagonists: Current status. Curr Med Chem 1994;1:313-345
9. Bradwejn J, Koszycki D, Meterissian G. Cholecystokinin-tetrapeptide induces panic attacks in patients with panic disorder. Can J Psychiatry 1990;35:83-85.
10. Evans BE, Rittle KE, Bock MG, DiPardo RM, Freidinger RM, Whitter WL, Lundell GF, Veber DF, Anderson PS, Chang RS, Cerino DJ. Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. J Med Chem 1988;31:2235-2246.
11. Simpson K, Serpell M, McCubbins TD, Padfield NL, Edwards N, Markam K, Eastwood D, Block R, Rowbotham DJ. Management of neuropathic pain in patients using a CCK antagonist Devazepide as an adjunct to strong opioids. 4th International conference on the mechanisms and treatment of neuropathic pain, San Francisco, USA, 2001.
12. Kitchen I, Crowder I. Assessment of the hot-plate antinociceptive test in mice: A new method for the statistical treatment of graded data. J Pharmacol Methods 1985;13:1-7.
13. Lattmann E, Billington DC, Poyner DR, Howitt SB, Offel M. Synthesis and evaluation of Asperlicin analogues as non-peptidal Cholecystokinin-antagonists. Drug Des Discov 2001;17:219-230.
14. Lattmann E, Billington DC, Poyner DR, Arayarat P, Howitt SB, Lawrence S, Offel M. Combinatorial solid phase synthesis of multiply-substituted 1,4-benzodiazepines and affinity studies on the $CCK_2$ receptor (Part 1). Drug Des Discov 2002;18:9-21.
15. Lattmann E, Sattayasai J, Billington DC, Poyner DR, Puapairoj P, Tiamkao S, Airarat W, Singh H, Offel M. Synthesis and evaluation of N1-substituted-3-propyl-1,4-benzodiazepine-2-ones as cholecystokinin ($CCK_2$) receptor ligands. J Pharm Pharmacol 2002;54:827-834.
16. Lattmann E, Singh H, Boonprakob Y, Lattmann P, Sattayasai J. Synthesis and evaluation of *N*-(3-oxo-2,3-dihydro-1H-pyrazol-4-yl)-1H-indole-carboxamides as cholecystokinin antagonists. J Pharm Pharmacol 2006;58:393-401.
17. Offel M, Lattmann P, Singh H, Billington DC, Bunprakob Y, Sattayasai J, Lattmann E. Synthesis of substituted 3-anilino-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepine-2-ones and their evaluation as cholecystokinin-ligands. Arch Pharm (Weinheim) 2006;339:163-173.
18. Yu Y, Jawa A, Pan W, Kastin AJ. Effects of peptides, with emphasis on feeding, pain, and behaviour. Peptides 2004;25:2257-2289.
19. Lattmann E, Sattayasai J, Boonprakob J, Lattmann P, Singh H. Synthesis and evaluation of *N*-(5-methyl-3-oxo-1,2- diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-N'-phenylureas as cholecystokinin antagonists. Arzneimittelforschung 2005;55:251-258.
20. Martin IL, Lattmann E. Benzodiazepine recognition site ligands and GABAA receptors. Expert Opin Ther Pat 1999;9:1347-1358.
21. Farghaly AM. Synthesis of some substituted aminophenazones of possible therapeutic interest. Pharmazie 1979;34:70-73.
22. Roth HJ, Kleemann A. Drug synthesis. Pharmaceutical Chemistry (Ellis Horwood). 1999;1:202-205
23. Sternbach LH, Fryer RI, Stempel A. Quinazolines and 1,4-Benzodiazepines. V. o-Aminobenzophenones. J Org Chem 1962;27:3781-3788.
24. Evans BE, Rittle KE, Bock MG, Freidinger RM. Design of nonpeptidal ligands for a peptide receptor: cholecystokin antagonists. J Med Chem 1987;30:1229-1239.
Charpentier B, Pelaprat D, Durieux C, Dor A, Reibaud M, Blanchard JC, Roques BP. Cyclic cholecystokinin analogues with high selectivity for central receptors. Proc Natl Acad Sci U S A 1988;85:1968-1972.
25. Scheibye S, Pederson BS, Lawesson SO. Dithiadiphosphetane and their application for organic syntheses. Bull Soc Chim Belg 1978;87:227-232.
26. Semple G, Ryder H, Kendrick DA, Szelke M, Ohta M, Satoh M, Nisida A, Akuzawa S, Miyata K. Synthesis and biological activity of 1-alkylcarbonyl-methyl analogues of YM022. Bioorg Med Chem Lett 1996;6:51-54.
27. Semple G, Ryder H, Kendrick DA, Szelke M, Ohta M, Satoh M, Nisida A, Akuzawa S, Miyata K, Rooker DP, Batt AR. (3R)-*N*-(tert-Butyl-carbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1H-1,4-benzodiazepin-3-yl)-*N*'-(3-(methyl-amino)phenyl)urea (YF476): A potent and orally active gastrin/CCK-B antagonist. J Med Chem 1997;40:331-341.
28. O'Neil MF, Dourish CT, Iversen SD. Morphine induced analgesia in the rat paw pressure test is blocked by CCK and enhanced by CCK antagonist MK-329. Neuropharmacology 1989;28:243-247.
29. Vogel HG, Vogel WH. Pharmacological assays. Drug discovery and evaluation (Springer) 1997; pp232-239
30. MattoV, Harro J, Allikmet L. The effects of drugs acting on the CCK receptors and rat exploration in the exploration box. J Physiol Pharmacol 1997;48:239-251.
31. Johnson NJ, Rodgers RJ. Ethological analysis of cholecystokinin (CCK-A and CCK-B) receptor ligands in the elevated plus maze in mice. Psychopharmacology (Berl) 1996;124:355-364.
32. Kilfoil T, Michel A, Montgomery D, Whithing RL. Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. Neuropharmacololy 1989;28:901-905.
33. Silverman P. Approach to a conditioned stimulus mazes. Animal behaviour in the laboratory (Chapman and Hall). 1978; pp110-119.
34. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730-732.
35. Walker JM, Dixon WC. A solid state device for the measuring sensitivity to thermal pain. Physiol Behav 1983;30:481-483.
36. Cowan A. Modern methods in pharmacology: Recent approaches in the testing of analgesics in animals. Testing and Evaluation of Drugs of Abuse (Wiley-Liss Inc.) 1990; pp21-33.
37. Speth RC, Wastek GJ, Johnson PC. Benzodiazepine receptors: Temperature dependence of [$^3$H]flunitrazepam binding. Life Sci 1979;24:351-357.